Human EGFR (Total) Singleplex Bead Kit Technical data Sheet

Catalog #: LHR9061 **Lot #:*** A10263

*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

Intended Use

This kit is comprised of components for the measurement of EGFR in cell lysates. Buffer reagents needed to complete the reaction are sold separately under Catalog #LHB0002. These reagents are intended for use in the **Luminex 100™ or 200™ System** only.

Note: This kit uses a Co-Incubation Assay format. See the User Manual included in the Intracellular Protein Buffer Reagent Kit (Cat # LHB0002) for further information.

Before attempting to multiplex this kit with any other kits, please contact Technical Support.

Reagents Provided

Human EGFR

1. **Part #:** LM197 **Description:** Antibody Bead Concentrate (10X) **Lot:** A10236 **Size:** 0.25 mL-100 tests

Bead Region: 68
Form: 0.25 mL 10x bead concentrate solution in storage buffer. Contains 0.05% sodium azide as a preservative.
Storage: **Light sensitive material.** Store at 2 - 8°C, in the dark, until the expiration date indicated on the kit.

Human EGFR (Total)

2. **Part #:** DN197 **Description:** Detection Ab Concentrate (10x) **Lot:** A10235 **Size:** 0.50 mL-100 tests

Form: 1.0 mL of a 10x stock of Detection Antibody Concentrate. Contains 0.1% sodium azide as preservative. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Detection Antibody.
Storage: Store at 2 - 8°C until the expiration date indicated on the kit.

3. **Part #:** SM057 **Description:** RTK Total 5-plex Standard **Lot:** S012505 **Size:** 2 Vials

Form: This standard (lyophilized recombinant proteins) is designated in ng/mL. Contains 0.1% sodium azide as preservative. The proteins in this standard have been calibrated with their respective Invitrogen ELISA kits. Detailed information on calibration is provided below.

Storage: Store at 2 - 8°C. Use within 1 hour after reconstitution. The remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. The standard can be frozen and thawed one time without loss of immunoreactivity.

Concentration of Reconstituted Standards:**

IR (15 ng/mL)	c-Kit (0.175 ng/mL)	IGF-1R (1.25 ng/mL)
c-Met (17.5 ng/mL)	EGFR (15 ng/mL)	

****Important note: The concentrations of reconstituted standards are lot-specific. Please verify all concentration values entered in data analysis software.**

Reconstitution: Reconstitute in **2.0 mL** *Assay Diluent*.

Recommended Starting Concentration for Standard Curve: Upon reconstitution, the starting concentration of standards is the value cited above. Make 2-fold serial dilutions in *Assay Diluent*. Refer to the User Manual included in the kit for further information.

Calibration: All markers in this reagent set were calibrated to their respective Invitrogen ELISA kits (catalog numbers): IR (KHR9111), IGF-1R (KHO0491), EGFR (KHR9061), c-Kit (KHO0391), and c-Met (KHO0251). Subsequent lots of standard will be normalized to this lot of material to allow consistency of quantitation.

Species Reactivity: This reagent set recognizes human proteins. Other species have not been tested.

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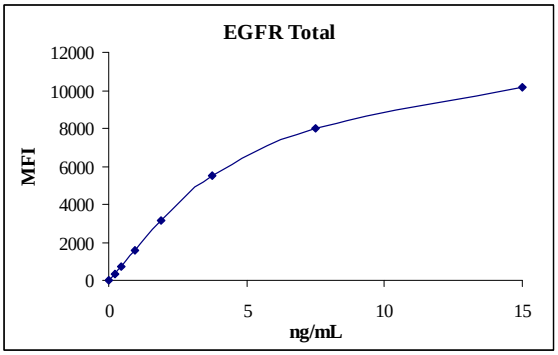
Human EGFR (Total) Singleplex Bead Kit

Technical data Sheet

Performance Characteristics

Typical Standard Curve



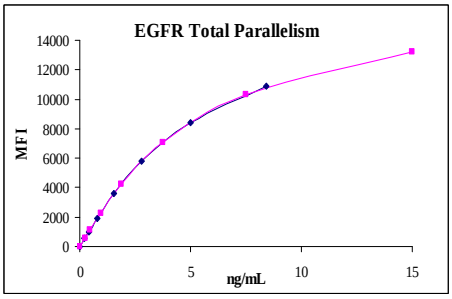
Note: This is a representative standard curve.
Absolute MFI values will vary.

Protein	EGFR Total
Sensitivity (ng/mL)[1]	0.02
Recovery (10% FNN0011)[2]	85.9
Recovery (10% FNN0021)[2]	94.8
Intra-assay variation (n=16)	5.35
Inter-assay variation (n=32)	5.83

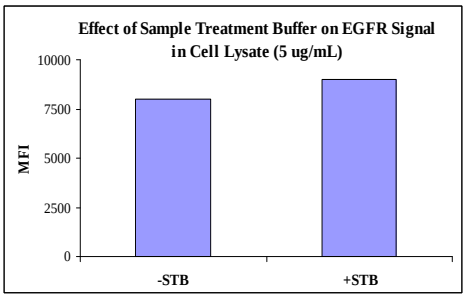
[1]**Analytical Sensitivity**: The analytical sensitivity was determined by adding two standard deviations to the median fluorescence intensity obtained when the zero standard was assayed 32 times.
[2]**Recovery**: To evaluate recovery, analytes were spiked at 3 different concentrations into 10% Cell Extraction Buffer (Cat. # FNN0011) and 10% NP40 Cell Lysis Buffer (Cat. # FNN0021).

Sample Preparation: This kit has been validated with cell lysates prepared in Cell Extraction Buffer (Cat. # FNN0011) and NP40 Cell Lysis Buffer (Cat. # FNN0021). Optimization of cell stimulation and cell lysis procedures may be required for each specific application. Refer to User Manual included in the Buffer Reagent Kit for detailed instructions.

Example Data: To evaluate the performance of this kit, natural EGFR from A431 cell lysate was serially diluted in *Assay Diluent*. The median fluorescence intensity of each dilution was plotted against the EGFR standard curve. Parallelism is presented in the figure to the right and indicates that the standard accurately reflects EGFR content in samples.



The data presented to the right demonstrate the impact of the *Sample Treatment Buffer* pre-incubation step on the observed signal. In this study, A431 cells were lysed in Cell Extraction Buffer. Lysates were either treated with *Sample Treatment Buffer* (+STB) or the *Sample Treatment Buffer* incubation step was omitted (-STB).



Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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